

## Supplementary Information

# A light-inducible CRISPR/Cas9 system for control of endogenous gene activation

Lauren R. Polstein<sup>1</sup> and Charles A. Gersbach<sup>1,2,3\*</sup>

<sup>1</sup>Department of Biomedical Engineering, Duke University, Durham, North Carolina, United States of America, 27708

<sup>2</sup>Center for Genomic and Computational Biology, Duke University, Durham, North Carolina, United States of America, 27708

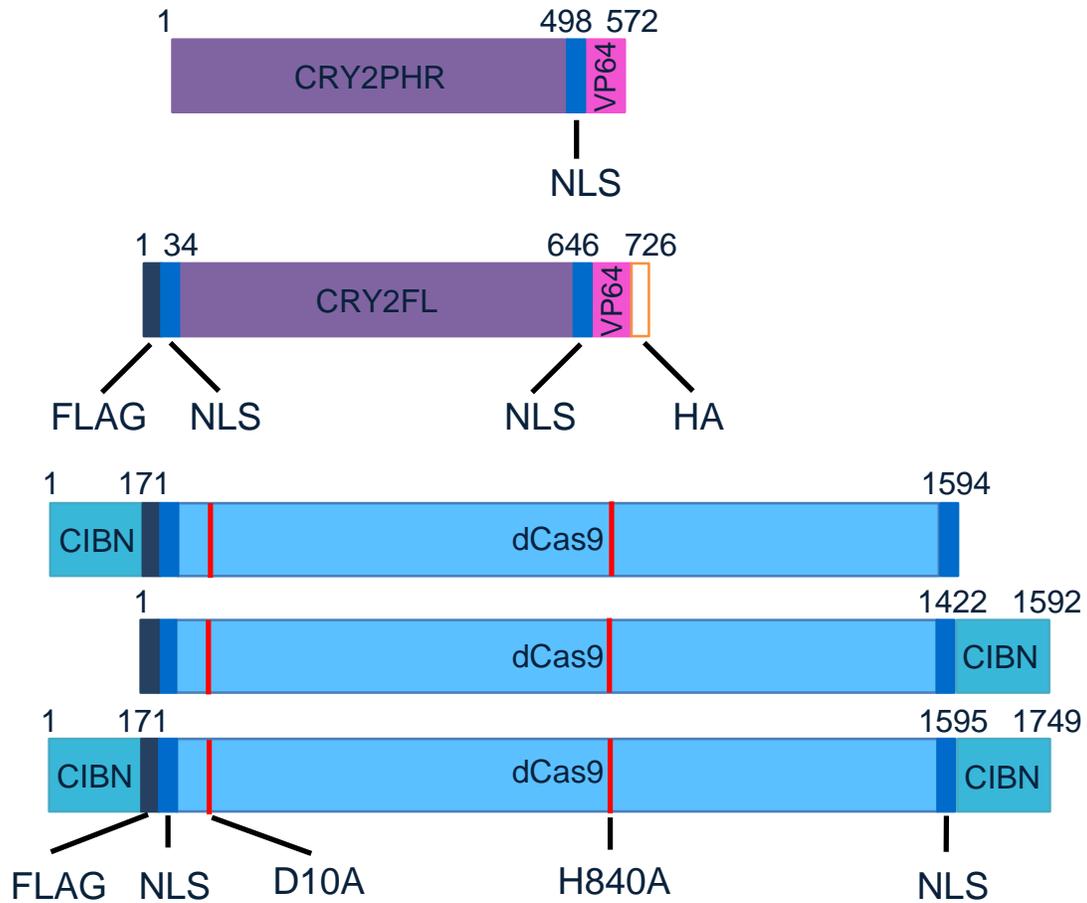
<sup>3</sup>Department of Orthopaedic Surgery, Duke University Medical Center, Durham, North Carolina, United States of America, 27710

\* Address for correspondence:

Charles A. Gersbach, Ph.D.  
Department of Biomedical Engineering  
Room 136 Hudson Hall, Box 90281  
Duke University  
Durham, NC 27708-0281  
Phone: 919-613-2147  
Fax: 919-668-0795  
Email: [charles.gersbach@duke.edu](mailto:charles.gersbach@duke.edu)

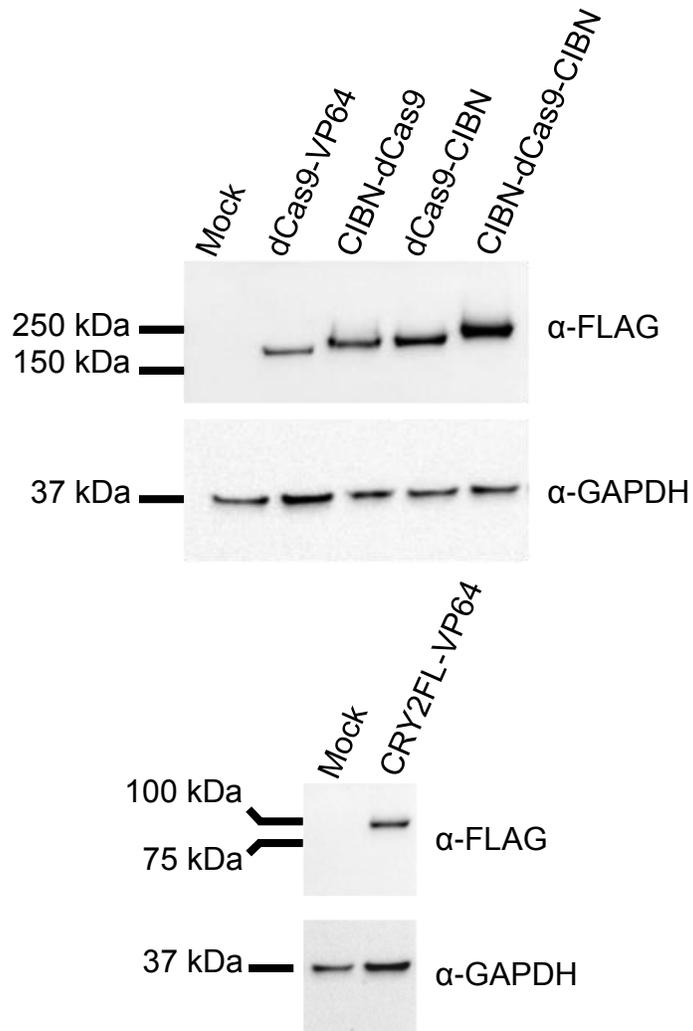
## **Supplementary Results**

All raw data for Figures 1 and 2 are included as a Microsoft Excel spreadsheet in the **Supplementary Dataset**.

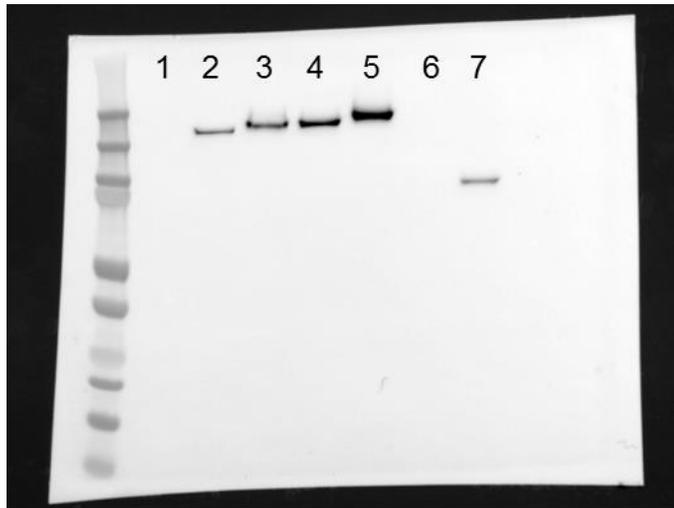


**Supplementary Figure 1.** Constructs tested to optimize the LACE system. Top panel, the N-terminal fragment of CRY2 (CRY2PHR, as previously described<sup>1</sup>) or full-length CRY2 (CRY2FL) is fused to the VP64 transactivation domain. CRY2FL-VP64 contains two SV40 NLS sequences and an N-terminal FLAG tag. Bottom panel, dCas9 was fused to the N-terminal fragment of CIB1 (CIBN) at its N-, C-, or N- and C- terminus. Each construct contains a FLAG tag at the N-terminus of dCas9 and two SV40 NLS sequences.

CRY2FL-dCas9 and CIBN-VP64 constructs were assembled and tested together, but did not result in activation of gene expression for unknown reasons.



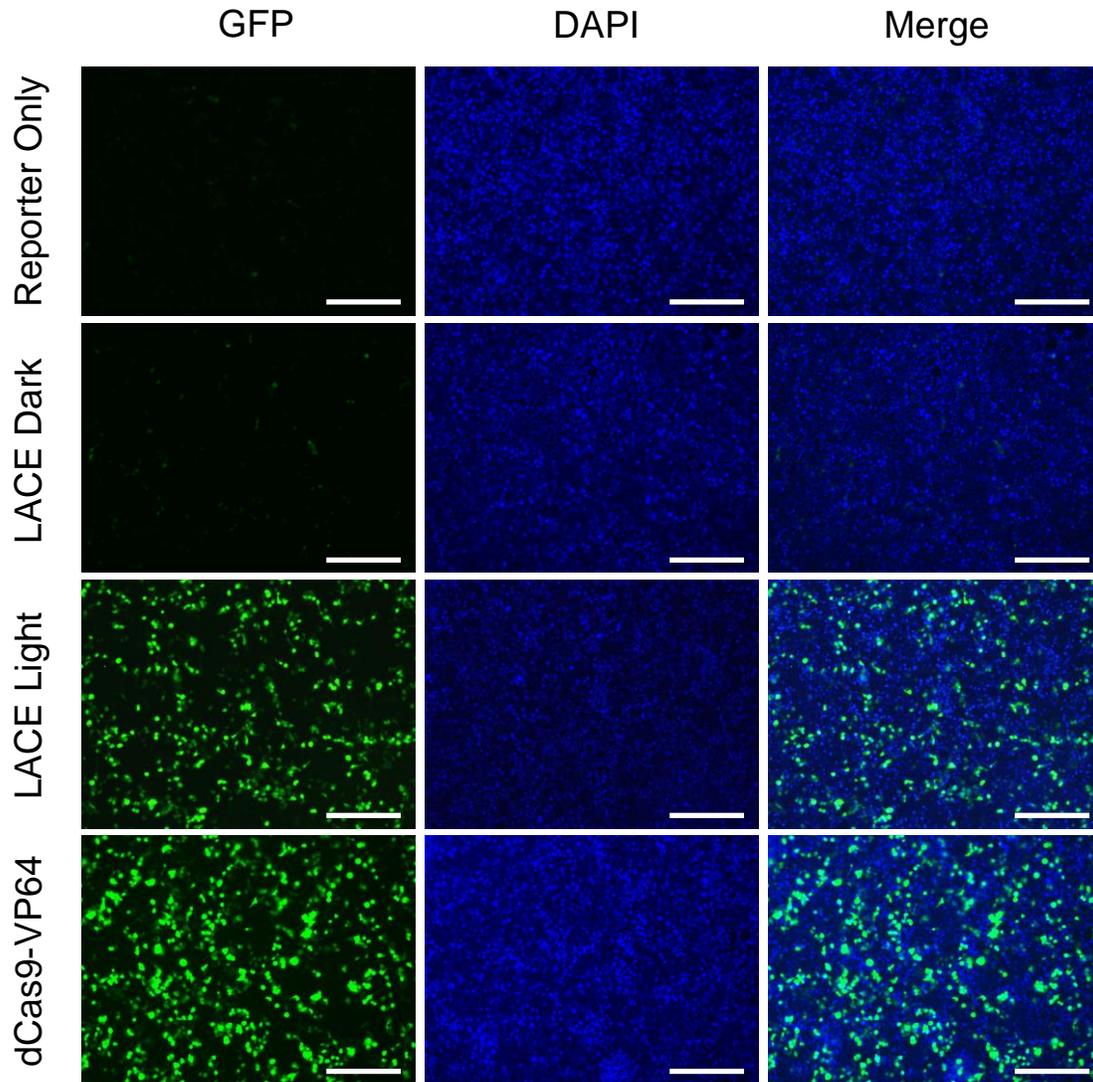
**Supplementary Figure 2.** Western blot of proteins expressed from LACE constructs transfected into human HEK293T cells. Constructs were probed with an anti-FLAG and anti-GAPDH was used as a loading control.



1. Mock
2. dCas9-VP64
3. CIBN-dCas9
4. dCas9-CIBN
5. CIBN-dCas9-CIBN
6. Mock
7. CRY2FL-VP64



**Supplementary Figure 3.** Un-cropped Western blot images from Supplementary Fig. 2 (top, anti-FLAG; bottom, anti-GAPDH).



**Supplementary Figure 4.** HEK293T cells were co-transfected with the LACE system or dCas9-VP64, an eGFP reporter, and a gRNA that targeted 8 identical DNA sites upstream of eGFP and a minimal CMV promoter. Cells that received the LACE system were either illuminated or incubated in the dark for 24 hours. Cells were transfected with the eGFP reporter and an empty plasmid as a negative control. Green = GFP, blue = DAPI. Scale bar = 200  $\mu$ m.

**Supplementary Figure 5.** Amino acid sequences of all proteins.

*dCas9-CIBN:*

MDYKDHDGDYKDHDIDYKDDDDKMAPKPKKRKVG<sup>RG</sup>MDKKYSIGLAIGTNSVGVAVITDEYKVP  
SKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVD  
DSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHA  
MIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIA  
QLPGEKKNLFGNLIASLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAA  
KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA  
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLRELLRQRTFDNGSIPHQIHLGELHAILRRQE  
DFYPFLKDNREKIEKILTRIPYYVGPLARGNSRFAMTRKSEETITPWNFEVVVDKGASAQSFIE  
RMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK  
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHLLKIIKDKDFLDNEENEDILEDIVLTLTLF  
EDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR  
NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKILQTVKVVDELVKVMGRHKP  
NIVIEMARENQTTQKQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDM  
YVDQELDINRLSDYDVAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLL  
NAKLITQRKFDNLTKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREV  
KVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVG<sup>TALIKKYPKLESEFVYGDYKVYDVR</sup>  
KMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRK  
VLSMPQVNIKKTEVQTTGGFSKESILPKRNSDKLIARKKDWDPKKG<sup>G</sup>FDSPTVAYSVLVAKVE  
KGKSKKLKSVKELLGITIMERSSEKPNIDFLEAKGYKEVKKDLI<sup>IKL</sup>PKYSLFELENGRKRMLASA  
GELQKGNELALPSKYVNFLYLASHYEKLKSPEDNEQKQLFVEQH<sup>KHYL</sup>DEIIEQISEFSKRVILAD  
ANLDKVL<sup>SAY</sup>NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ  
SITGLYETRIDLSQLGGDPIAGSKASPKPKKRKVG<sup>RATM</sup>NGAIGG<sup>DLLN</sup>FDPMSVLERQRAHLKYL  
NPTFDSPLAGFFADSSMITGGEMDSYLS<sup>TAGLN</sup>LPMMYGETTVEGDSRLSISPETTLGTGNFKKRK  
FDTETKDCNEKKKKMTMNRDDLVEEGEEEEKSKITEQ<sup>NN</sup>GSTKSIKKMKHKAKKEENNFSNDSSK  
VTKELEKTDYI\*

FLAG tag  
SV40 NLS  
dCas9  
CIBN

*CIBN-dCas9:*

MDYKDHDGDYKDHDIDMNGAIGGDLNFPDMSVLERQRAHLKYLNPTFDSPLAGFFADSSMIT  
GGEMDSYLSTAGLNLPMYGETTVEGDSRSLSPETTLGTGNFKKRKFDTETKDCNEKKKKMTM  
NRDDLVEEGEEEEKSKITEQNGSTKSIKKMKHKAKKEENNFSNDSSKVTKELEKTDYIHHDYKDD  
DDKMAPKKRKRKVGGRGMDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA  
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVD  
KLFQVLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTP  
NFKSNFDLAEDAQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAP  
LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM  
DGTEELLVKNLREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPY  
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSL  
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEI  
SGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKV  
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ  
VSGQGDSLHEHIANLAGSPAIKKILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNS  
RERMKRIEELGKELGSQLKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVAIVP  
QSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG  
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFY  
KVREINNYHHAHDAYLNAVVGTAALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFY  
SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKTEVQTGGF  
SKESILPKRNSDKLIARKKDWDPKKGFFSPTVAYSVLVAKVEKKGSKKLKSVKELLGITIMER  
SSFENPIDFLEAKGYKEVKKDLIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFYLYL  
ASHYEKLGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHARDKPIRE  
QAENIHLFTLNLGAPAAFYFDTTIDRKRYTSTKEVLDTLHQSITGLYETRIDLSQLGGPIAG  
SKASPKKKRKRKVGRA\*

FLAG tag  
SV40 NLS  
dCas9  
CIBN

*CIBN-dCas9-CIBN*

MDYKDHDGDYKDHDIDMNGAIGGDLNFPDMSVLERQRAHLKYLNPTFDSPLAGFFADSSMIT  
GGEMDSYLSTAGLNLPMMYGETTVEGDSRLSISPETTLGTGNFKKRKFDTETKDCNEKMKMTM  
NRDDLVEEGEEKSKITEQNNGSTKSIKMKHKAKKEENNFSNDSSKVTKELEKTDYIHHDYKDD  
DDKMAPKKRKVGRGMDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA  
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERH  
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVD  
KLFQVLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGNLIASLGLTP  
NFKSNFDLAEDAQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAP  
LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM  
DGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTRIPY  
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSL  
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEI  
SGVEDRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKV  
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ  
VSGQGDSLHEHIANLAGSPAIKKILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNS  
RERMKRIEELGKELGSQLKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVAIVP  
QSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG  
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFY  
KVREINNYHHAHDAYLNAVVGTAALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFY  
SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKTEVQTGGF  
SKESILPKRNSDKLIARKKDWDPKKGFFSPTVAYSVLVAKVEKKGSKKLKSVKELLGITIMER  
SSFENPIDFLEAKGYKEVKKDLIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFYLYL  
ASHYEKLGSPEDNEQKQLFVEQHKHYLDEIEQISEFSKRVLADANLDKVL SAYNKHARDKPIRE  
QAENIHLFTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLHQSIITGLYETRIDLSQLGGPIAG  
SKASPKKKRKVGRATMNGAIGGDLNFPDMSVLERQRAHLKYLNPTFDSPLAGFFADSSMITGG  
EMDSYLSTAGLNLPMMYGETTVEGDSRLSISPETTLGTGNFKKRKFDTETKDCNEKMKMTMNR  
DDLVEEGEEKSKITEQNNGSTKSIKMKHKAKKEENNFSNDSSKVTKELEKTDYI\*

FLAG tag  
SV40 NLS  
dCas9  
CIBN

*CRY2FL-VP64*

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGRMKMDKKTIVWFRRDLRIEDNPALAAAAH  
EGSVFPVFIWCPEEEGQFYPPGRASRWWMKQSLAHLSQLKALGSDLTLIKTHNTISAILDCIRVTG  
ATKVVFNHLYDPVSLVRDHTVKEKLVERGISVQSYNGDLLYEPWEIYCEKGGKPFSTFNSYWKKCL  
DMSIESVMLPPPWRMLPITAAAEAIWACSIIEELGLENEAEKPSNALLTRAWSPGWSNADKLLNEF  
IEKQLIDYAKNSKKVVGNSTSLLSPYLHFGEISVRHVFCARMKQIIWARDKNSEGEESADLFLRGI  
GLREYSRYICFNFPFTHEQSLLSHLRFFPWDADVDKFKAWRQGRGTGYPLVDAGMRELWATGWM  
HNRIRVIVSSFAVKFLLLPWKWGMKYFWDTLDDADLECDILGWQYISGSIPDGHELDRLDNPALQ  
GAKYDPEGEYIRQWLPELARLPTEWIHPWDAPLTVLKASGVELGTNYAKPIVDIDTARELLAKA  
ISRTREQIMIGAAPDEIVADSFEALGANTIKEPGLCPSVSSNDQQVPSAVRYNGSKRVKPEEEEEER  
DMKKSRRGFDERELFSTAESSSSSVFFVSQSCSLASEGKNLEGIQDSSDQITTSLGKNGCKPIAGSKA  
SPKKKRKVGRADALDDFDLMLGSDALDDFDLMLGSDALDDFDLMLGSDALDDFDLMLIN  
YPYDVPDYAS\*

FLAG tag  
SV40 NLS  
CRY2FL  
VP64

**Supplementary Figure 6.** DNA sequence of the promoter for the eGFP reporter.

*8x gRNA-eGFP reporter*

CTGCAAAGGTCGAGAACTGCAAAGGTCGAGAACTGCAAAGGTCGAGAACTGCAAAGG  
TCGAGAACTGCAAAGGTCGAGAACTGCAAAGGTCGAGAACTGCAAAGGTCGAGAACT  
GCAAAGGTCGAGAACTGCAAAGAGATCTGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC  
AGAGCTCGTTTAGTGAACCGTCAGATCAAGCTTGGCATTCCGGTACTGTTGGTAAAGCCACcatgG  
TGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTA  
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCT  
GAAGTTCATCTGCACCACCGGCAAGCTGCCCCTGCCCTGGCCCACCCTCGTGACCACCCTGACCTA  
CGGCGTGCAGTGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCA  
TGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC  
GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA  
GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCA  
TGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGC  
AGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGGCGACGGCCCCGTGCTGCTGCC  
GACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACAT  
GGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

8x gRNA binding sites

Minimal CMV promoter

eGFP

## Supplementary References

1. Konermann, S., Brigham, M.D., Trevino, A.E., Hsu, P.D., Heidenreich, M., Cong, L. et al. Optical control of mammalian endogenous transcription and epigenetic states. *Nature* **500**, 472-476 (2013)